J Clin Pharmacol 1985;25:164–175

Chemoprevention of Cancer: Implications for Clinical Pharmacology

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Cancer chemoprevention may be defined as the prevention of cancer in human populations by chemical agents that inhibit carcinogenesis. The concept of cancer chemoprevention is based on the cancer inhibitory potential of certain chemical compounds that may be considered as cancer chemopreventive agents. The characteristics of cancer chemopreventive agents that are relevant to human cancer prevention include their mechanisms of action, toxicity, and efficacy. A theoretic approach to selection of cancer chemopreventive agents for human clinical trials is presented. Since cancer chemopreventive agents currently used in clinical trials include micronutrients and their synthetic analogues, the cancer preventive activity of this class of agents is specifically reviewed.

CANCER PREVENTION AND CLINICAL PHARMACOLOGY

Cancer Prevention

Preventive medicine offers considerable opportunities for the improvement of human health while confronting the realities of increasingly limited health resources. Scientific advances have also led to the recognition that many chronic diseases may be preventable. Cancer is no longer considered an integral component of the aging process. Environmental factors, acting in the presence of possible genetic factors, are now recognized as important determinants of human cancer. These environmental determinants may include such factors as dietary habits and lifestyle.

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Cancer prevention may focus on several levels in the effort to reduce cancer incidence and mortality in human populations. For example, reduction in exposure to environmental risk factors may reasonably be expected to reduce the incidence of many human cancers. Within the United States, it has been estimated that 80% to 90% of cancer incidence may be attributable to environmental risk factors, and that dietary and nutritional factors may account for approximately 35% of attributable risk for cancer in human populations.[1,2] Other risk factors such as smoking and various occupational exposures to industrial carcinogens may also be readily recognized. Strategies for recognition of populations at high risk of cancer may result in effective allocation of health resources, and may also create special opportunities for cancer prevention. If different cancer risk factors are identified, various interventions among high risk groups may act to prevent cancer before it has developed.

There is considerable evidence for the role of chemical carcinogenesis in human cancer and increasing evidence that this process can be reversed if the chemical insult is removed or neutralized. Carcinogenesis induced by chemicals involves the separate and independent processes of tumor initiation and tumor promotion. Initiation causes molecular or cellular alterations that may lead to cancer or may remain dormant in the absence of a promoter. A promoter may induce cellular proliferation, leading to expansion of clones of initiated cells.[3]

Mutations in cells caused by initiation appear to be irreversible, while the slow process of promotion may be reversed or slowed to the point of never reaching tumor production as the end point of carcinogenesis. The initiation and promotion processes are consistent with a multistage model of carcinogenesis, in which progressive neoplastic enhancement of cells occurs through a series of carcinogenic events in populations of cells exposed to carcinogens. Several such rare events in succession are thought to be necessary in

order to transform a population of normal cells into malignant cells. These stages of cell transformation may be observable histopathologically.[4] Understanding the multistage initiation and promotion processes and their potential for inhibition may be a key to cancer prevention.

Numerous mechanisms for the chemical inhibition of carcinogenesis have been proposed.[5] Identification of agents that may inhibit the multistage process of carcinogenesis and prevent the occurrence of neoplasms is important to cancer prevention. Given the background of modern medical science and therapeutic expectation, it may be easier or more appropriate in some situations to prescribe preventive agents than to proscribe unhealthy diets and other practices among human populations. For example, prevention of cancer in humans may be more readily achieved by prescription of a specific micronutrient than by attempting substantial alterations in human behavior involving major modifications of the human diet.

Chemical compounds that inhibit carcinogenesis may be considered as cancer chemopreventive agents. Such chemical agents may be used to prevent cancer in high risk groups or in the general population. Therefore, cancer chemoprevention may be defined as prevention of cancer in human populations by chemical agents that inhibit carcinogenesis. As such, cancer chemoprevention should be considered a component of primary cancer prevention because it acts prior to the development of disease. Cancer chemopreventive agents may be identified within a broad spectrum of chemical compounds that (1) induce detoxification of carcinogens, (2) scavenge reactive carcinogen species, (3) induce cellular differentiation, and (4) reverse any other reversible preneoplastic process.

Application of Clinical Pharmacology

The characteristics of cancer chemopreventive agents that are relevant to human cancer prevention include their structure/activity relationships, mechanisms of action, toxicity, efficacy, dose, and dosage form. Selection of cancer chemoprevention agents for human cancer prevention involves in vitro and in vivo screening for efficacy, preclinical toxicity and pharmacokinetic studies, and clinical studies in humans as well as clinical dosage form development. A wide range of chemical compounds have been noted to manifest cancer inhibitory potential. These observations indicate that structurally related compounds, or their active moieties, may have similar cancer inhibitory potential; and they further suggest avenues for development of future cancer chemopreventive agents. Cancer chemopreventive agents that may inhibit later stages in the multistage process of carcinogenesis may be particularly useful for preventing cancer in human populations already exposed to carcinogens.

An important characteristic of cancer chemopreventive agents is low toxicity, since the potential target population for cancer prevention includes individuals who are not ill, but only at risk of disease, and may also include individuals with only a "normal" risk of exposure to environmental carcinogens. The requirement for low toxicity of chemopreventive agents for cancer prevention is in contrast to the typical features of chemotherapeutic agents for cancer therapy. Clinical pharmacology has played an important role in the development, screening, identification, and testing of chemotherapeutic agents.[6,7] Since identification of cancer chemopreventive agents is a current component of cancer prevention, chemoprevention of cancer presents an exciting new field for clinical pharmacology.

It has been recognized that chemical inhibitors of carcinogenesis may be synthetic compounds or may occur naturally in foods as components of the human diet. Cancer chemopreventive agents in the diet may be micronutrients or other metabolically active constituents or food additives. Micronutrients (e.g., vitamins and trace elements) are present in the human diet in low concentrations and mediate critical aspects of human biochemistry and metabolism. Synthetic analogues of micronutrients, which may have equal or greater efficacy (and less toxicity) than the parent compound, have also been recognized as potential chemical inhibitors of carcinogenesis and are of particular interest for use as cancer chemopreventive agents.

POTENTIAL FOR CHEMICAL INHIBITION OF CARCINOGENESIS

The potential for chemical inhibition of carcinogenesis by chemopreventive agents has been suggested both by animal experimental evidence and human epidemiologic studies. Early studies on chemical inhibition of carcinogenesis in animals were conducted as a result of clinical and epidemiologic observations on cancer in humans.[8] The dramatic geographic and ecologic variations in cancer patterns, and changing

incidence of cancer in migrants, point to differential exposure of human populations to cancer risk factors and/or cancer protective factors. Dietary factors almost certainly play a prominent role in explaining these differing cancer rates. As a result of these and other observations, a full range of investigations are being conducted, extending from animal studies in the laboratory to analytic epidemiology in human populations. However, a human clinical trial is the ultimate test of efficacy of a cancer chemopreventive agent for cancer prevention in humans.

The potential for chemical inhibition of carcinogenesis by chemopreventive factors in the diet will be considered in the context of (1) human epidemiologic evidence, interpretation, and limitations, and (2) human intervention studies.

Human Epidemiologic Evidence, Interpretation, and Limitations

Observational epidemiology, involving both descriptive (correlation) and etiologic (case-control and prospective cohort) studies of cancer, helps to identify factors that inhibit carcinogenesis. Correlation studies generally estimate the relationship between environmental factors and cancer frequencies. Cross-sectional analyses of international differences in cancer incidence and mortality rates indicate the importance of environmental factors in the etiology of cancer.[9-15] Breast cancer rates, for example, are five times higher in the United States than in Japan. These differences are not explained by genetic factors, as epidemiologic studies of Japanese migrants to the United States demonstrate a higher incidence of breast cancer in migrants than in Japanese living in Japan.[11,16-18] As migrants undergo progressive acculturation to a western life-style,[19] breast cancer rates in migrants progress toward those in the host country. It has been observed that persons who emigrate during childhood and adolescence experience the greatest degree of changes in cancer patterns.[17] It is often not possible to pinpoint the environmental factor(s) responsible for observed differences in cancer patterns. However, results of these international correlation studies suggest that exposure to different environmental carcinogens and/or cancer promoters or protective factors varies greatly among different human populations around the world. These crude observations on populations are not definitive tests of cancer chemopreventive agents, but are useful in generating hypotheses for further testing.

Case-control studies conducted in a retrospective manner, and cohort studies conducted prospectively, are more specific approaches to identifying potential cancer chemopreventive agents. Unlike correlation analyses that are generally limited to a few variables known only at the population level, case-control and cohort approaches study individuals and usually obtain information on a large number of different variables. In these studies, information on exposure to potential cancer protective factors is determined through dietary assessment[20] or direct measurement of biologic specimens.[21-23] While several potential chemopreventive agents have been identified by these approaches,[24-26] these studies also have limitations.

The human diet is varied and complex and can not be easily assessed or simply characterized. The precision and accuracy of information collected by dietary interview is limited by the human capacity for recall. Diet records or diaries are laborious to maintain and can only be collected on a limited number of individuals over a brief period of time. True validation of long-term dietary intake is complicated and difficult, if not impossible.[27] Knowledge of their diagnosis among cancer patients in case-control studies may bias their dietary recall. The specific nutrient content of the foods that are reported to be ingested by individuals must also be determined. The accuracy of translation from foods to nutrients is dependent on the availability and reliability of food composition tables. Although these tables are constantly being revised, many of the values reported are out of date, incomplete, or too generalized to be useful.

While biologic samples generally provide more objective measurements of the intake of nutrients, their relevance is not always clear and they also have methodologic limitations. Adequate methods for measuring many potential chemopreventive agents are not yet available. The relation between dietary intake and nutrient levels in biologic samples is remote for many micronutrients, such as vitamin A (retinol). Moreover, samples are generally obtained at only one point in time for determination of nutrient levels in biologic fluids. Little or no data are available on the relationship of a single biologic measurement of one micronutrient at one point in time to long-term nutrient status or to the multistage process of carcinogenesis with respect to the timing of tumor development. If nutrient intake in early life is relevant to long-term cancer risk,[28] for example, case-control studies on elderly cancer patients may not be sufficient to illus-

trate the definitive role of nutrient intake in youth on the early stages of the multistage process of carcinogenesis.[15] Biologic measurements of nutrient levels on cancer patients in case-control studies may also be influenced by the effects of disease on the nutrient of interest.[29-31] For example, a low measurement for a potential chemopreventive agent in a patient with cancer may reflect an effect of the disease on the agent rather than a lack of protective effect of the agent on the disease.

Furthermore, attention must be paid to the particular chemical form or derivative of the nutrient or other agent that is measured in biologic specimens.[25,32] Potential cancer chemopreventive agents must be considered as chemical compounds. Although their presence in the human diet has led to recognition of the potential for chemical inhibition of carcinogenesis, definitive tests of cancer chemopreventive agents should be conducted under controlled conditions with the agent as the only variable differentiating the treated and control groups or in well-designed, multiagent studies.

Human Intervention Studies

Human intervention studies of potential cancer chemopreventive agents may be considered in three ways (1) the need for controlled clinical trials; (2) the scope and design of these trials; and (3) the requirements for pharmacologic and toxicologic information on these chemical compounds prior to their use in human clinical trials.

Observational epidemiology has been used to identify chemical compounds that may inhibit carcinogenesis, but this approach generally lacks specificity. Intervention research on potential cancer chemopreventive agents is a new and more definitive approach that provides evidence of efficacy unachievable in observational and analytic epidemiology. It is important both practically and theoretically to test the potential chemopreventive effects of chemical inhibitors of carcinogenesis in a rigorous fashion in humans. Large, randomized, placebo-controlled trials among individuals with no previous history of the disease are the most reliable manner in which to test directly whether a potential cancer chemopreventive agent prevents the development of human cancer.[33] In a clinical trial of a single micronutrient, randomization techniques are designed to achieve treatment groups that differ only in the specific micronutrient under study. In addition to testing the value of chemopreventive agents, such studies assist in resolving uncertainty about the relevance of animal models, questions about participant acceptance of the intervention, and the cost/benefit ratio of the intervention.[34]

The target population for such studies includes individuals who are free of the disease under study, but are at some risk of development of the disease. If a trial is to be conducted in essentially healthy individuals, this imposes the requirement of a large sample population to ascertain outcome. However, in a group at high risk of cancer the expected number of events to be observed may be significantly greater than in a low or normal risk group. Studying a high risk group, therefore, may be more efficient than studying a low risk group in that a larger number of cancer cases can be identified within a smaller population over a shorter period of time. Nonetheless, such studies are typically long, usually requiring three to five years in order to show an effect of the chemopreventive agent.

Intervention studies are controlled in that the study population is divided into two or more treatment groups by random assignment. However, more than one treatment or intervention may be tested in the same trial through a factorial design, in which multiple agents are given in various combinations. This consideration is important since the optimal cancer chemopreventive regimen may contain several different agents, depending on their mechanisms of action.

Before a potential cancer chemopreventive agents can be used in a human clinical trial, information must be acquired on the mechanisms of action, safety, and efficacy. Such information is initially established in the laboratory in animal models and in the test tube. For example, the efficacy of cancer chemopreventive agents may be initially established in vitro and in animal tumor systems in vivo. In vitro and in vivo screening for efficacy, if positive, is generally followed by specific evaluation of acute, subacute/subchronic, and chronic toxicity in animals. These methods are important components of clinical pharmacology that are applied directly in selection of cancer chemopreventive agents.

As chemical inhibitors of carcinogenesis, cancer chemopreventive agents have mechanisms of action that are related to basic human biochemistry and metabolism as well as mechanisms of carcinogenesis. The activity of potential cancer chemopreventive agents may be related to known mechanisms for

chemical inhibitors of carcinogenesis. Knowledge of these mechanisms is helpful to understanding the possible types of activities of cancer chemopreventive agents in clinical trials, and may relate to selection of appropriate agents by structure-activity relationships for future studies.

GENERAL MECHANISMS OF CHEMICALS THAT INHIBIT CARCINOGENESIS

There appear to be two basic mechanisms by which chemical agents with relatively low toxicity may inhibit carcinogenesis. The first mechanism acts by altering the manner in which the carcinogen is handled by the organism prior to the time it reacts with critical target sites. A second mechanism acts later by altering the biologic properties of cells that have already been subjected to the effects of chemical carcinogens in the multistage process of carcinogenesis. The mechanisms of action of agents that inhibit cancer initiation are based on the proposed metabolic processing of carcinogens that occurs in a step-wise fashion. Most carcinogens display ultimate reactive forms consisting of a positively charged electrophilic nucleus that chemically interacts with macromolecules such as DNA. Many carcinogens are metabolically activated to this form via oxidation by the microsomal mixed function oxidase system, or by other metabolic pathways. Other important carcinogens apparently do not require metabolic activation prior to manifesting an ability to cause initiation at the cellular level.[35]

Since many carcinogens are viewed as environmental chemicals that enter the body, metabolic processing of chemical carcinogens is relevant to carcinogenesis and cancer chemoprevention. Compounds that inhibit carcinogenesis may act at different steps in the metabolic processing of a carcinogen from procarcinogen to proximate carcinogen to ultimate carcinogen. A procarcinogen is a chemical absorbed into the metabolic system from the environment and is subsequently transformed into a proximate carcinogen. The proximate carcinogen is then metabolically converted into the ultimate carcinogen that displays its final carcinogenic activity at the molecular or cellular level. Thus, a chemopreventive agent may act to prevent formation or absorption of a procarcinogen, to modulate biotransformation in a manner that will prevent formation of ultimate carcinogens, to accelerate solubilization and excretion of procarcinogens and proximate carcinogens, and finally, to block the action of ultimate carcinogens once they are formed. Examples of each of these mechanisms of chemical inhibition of carcinogenesis are illustrated using agents with known chemopreventive activity.

Prevention of Formation or Absorption of Procarcinogen

The nitrite ion (or nitrous anhydride in lipid media) and amines or amides are present in food supplies and may interact with constituents in the gastrointestinal tract to produce carcinogenic nitrosamines and nitrosamides. The micronutrients vitamin C and vitamin E may act as a complementary pair to block the formation of nitrosamines and prevent absorption in the gastrointestinal tract. Vitamin C reduces the nitrite ion in aqueous media to nonreactive nitric oxide. Likewise, vitamin E reduces the counterpart nitrous anhydride in lipid media.[36]

Prevention of Ultimate Carcinogen Formation

Benzo(a)pyrene (BP) is an example of a class of carcinogenic polycyclic hydrocarbons found in automobile exhaust gases, cigarette smoke, and heat-charred meat. The cytochrome P450 mixed function oxidase system produces the ultimate carcinogenic form of BP by oxidizing a portion of its ring structure to an epoxide, which is intensely electrophilic and will bind to and distort the structure of DNA.[37] Disulfiram, a compound that inhibits forestomach tumor induction by BP in rats, produces a significant decrease in liver microsomal cytochrome P450 oxidase activity by combining with the active site of the enzyme. The formation of the BP epoxide is thereby reduced.

Acceleration of the Solubilization and Excretion of Procarcinogens and Proximate Carcinogens

Butylated hydroxyanisole (BHA), found in processed foods as a preservative, belongs to a class of chemopreventive compounds that operate by inducing a number of liver enzymes. Some of these liver enzyme systems catalyze the conjugation of carcinogens that increases their solubility, and thereby accelerates their excretion in the urine. One of these enzymes is glucuronyl transferase. Aryl hydrocarbon hydroxylase, a member of the P450 mixed function oxidase system, catalyzes addition of a hydroxyl group to the

aromatic ring structure of carcinogenic polycyclic aromatic hydrocarbons that are subsequently conjugated to glucuronic acid by glucuronyl tranferase. The conjugate is water soluble and is rapidly eliminated by the kidneys.

Blockage of Ultimate Carcinogens

Ultimate carcinogens are almost invariably strongly electrophilic[35] and they can be blocked from reacting with DNA by a competing nucleophile. Glutathione-S-transferase is an important liver enzyme that catalyzes the reaction between the strongly nucleophilic glutathione and the electrophilic site of an ultimate carcinogen, thereby rendering the carcinogen inactive. Butylated hydroxyanisole will induce as much as a tenfold increase in the activity of glutathione-S-transferase when added to the designated diet of rats.[38] This activity appears to explain why the butylated hydroxyanisole prevents tumors produced by a significantly broad range of carcinogens.

Mechanisms Based on the Theory of Free Radical Carcinogenesis

Beta-carotene, vitamin E, vitamin C, and selenium may produce an inhibitory effect on chemically induced tumors by mechanisms based on the theory of free radical carcinogenesis.[39] The activated oxygen species of hydrogen peroxide and superoxide are continuously produced in small amounts during mitochondrial respiration and also by the P450 mixed function oxidase system during the oxidation of xenobiotic compounds. The superoxide and hydrogen peroxide can react with each other in the presence of trace amounts of iron to produce hydroxyl free radicals that then initiate the free radical chain reactions such as lipid peroxidation. Through this process they eventually induce DNA alterations that can lead to cancer. Beta-carotene[40] and vitamin E[41] both function as effective radical-trapping antioxidants, thereby preventing free radical carcinogenesis. The tumor preventive action of selenium compounds depends in part on the fact that selenium is present in glutathione peroxidase. This enzyme, together with the superoxide dismutase, forms the very first line of defense against activated oxygen production under normal circumstances. An increased selenium intake leads to increased glutathione peroxidase activity in those populations with low selenium status.

Mechanisms Based on Later Phases in the Multistage Model of Carcinogenesis

There are a number of cancer inhibitory compounds for which the mechanism of action appears to operate later in the multistage process of carcinogenesis. Reversal of the early phases of neoplasia by chemical inhibitors requires alteration of the biologic properties of cells that have already been subjected to the effects of chemical carcinogens. Vitamin A and related compounds have received attention as potential cancer chemopreventive agents that may act by reversing cellular effects of early carcinogenic events. Retinoids, analogues of vitamin A, may act by inhibiting the tumor promotion phase or may inhibit tumor cell growth, with reversal of certain aspects of transformed cell phenotype (e.g., reversal of squamous metaplasia in epithelial cells). According to Sporn,[42] a tentative mechanism for retinoids may be that they suppress the effect of polypeptide hormones that are produced by tumors and act to enhance cell division. Retinoids also induce cell differentiation. Other possible mechanisms include the interaction of retinoids directly with the genome.

Potential cancer preventive compounds that operate by unknown mechanisms include aromatic isothiocyanates, certain methylated flavones, coumarins, and the prostaglandin synthesis inhibitors indomethacin and piroxicam. These general mechanisms of action of inhibitors of carcinogenesis may provide some understanding that may be relevant in selecting potential cancer chemopreventive agents for use in human intervention trials. A theoretic approach to selection of cancer chemopreventive agents for human intervention trials is described in the next section.

APPROACH TO HUMAN INTERVENTION TRIALS WITH CANCER CHEMOPREVENTIVE AGENTS

Existing known chemopreventive compounds have a wide range of chemical structures, suggesting by structure-activity relationships that other active compounds may exist within this spectrum.[5] Agents with potential chemopreventive activity may be initially identified through structural homology with agents having known chemopreventive activity, as in the development of synthetic analogues of micronutrients. Potential chemopreventive agents may also be identified by initial experimental findings in the

laboratory, observations in the clinical setting, or from descriptive or analytic epidemiologic studies.

Establishment of Efficacy

Systematic evaluation of potential agents identified through the above sources may include in vitro screening and evaluation to determine the presence and nature of inhibitory mechanisms at the cellular level; and in vivo screening to determine the level of cancer preventive activity in a biologic system.

In Vitro Screening

Several well-defined continuous cell line cultures and organ cell culture systems provide a valuable in vitro adjunct to the in vivo systems available for evaluation of potential chemopreventive agents.[43,44] The in vitro technologies provide certain distinct advantages over in vivo methodologies, including time/cost efficiency, sensitivity, ease of quantitation, and more controlled conditions with fewer variables. They also allow experimentation on human cells with agents that are not approved for human subjects. Such advantages are clear, but should be taken in proper perspective with the limitations of the in vitro methodologies.

The primary limitation of in vitro screening is the inability to determine the relevance of the observed in vitro activity to the potential efficacy (or toxicity) in the intact organism. The in vitro assays can, however, serve as efficient screens of potential chemopreventive activity against a variety of different mechanisms of transformation. The promising agents can subsequently be evaluated by in vivo screening assays where the relevant questions of bioavailability, tissue distribution, metabolism, and in vivo efficacy and toxicity can be determined.

A second significant limitation of the in vitro methods for evaluating chemopreventive agents is that the cell culture systems employed are primarily fibroblastic cell lines. Since at least 80% to 90% of human cancers are of epithelial origin, the fibroblastic systems would appear to impose a serious limitation. It is not clear that the phenotypic properties of these cells, and the results obtained using them, can be applied to epithelial cells. While certain well-accepted criteria for transformation of fibroblastic cells are shared by epithelial cells, other criteria are not as reliably measured in epithelial cells. Evaluation of chemopreventive agents may best be performed by utilizing (1) standard proved fibroblastic cell lines that show a strong irreversible promoter-dependent change in anchorage-independent growth or (2) human epithelial cell cultures that can be transformed in vitro by two-stage assays.[45] Other specialized cell lines provide useful markers associated with transformation. A battery of cell cultures may therefore be utilized to detect the different effects of chemopreventive agents.

In addition to continuous cell lines or special cell cultures, in vitro organ cultures have become important in recent years in the study of cancer causing agents and will become equally important in the study of chemopreventive agents. Chemopreventive agents (as well as the carcinogens and promoters that they inhibit) generally exhibit tissue specificity, and their effect can vary greatly with different species and exposure conditions. This tissue specificity suggests the importance of the adjunctive role that in vitro organ culture systems should play in the evaluation of chemopreventive compounds. Different mouse skin systems are well studied and provide prototype systems for this kind of evaluation. Promising systems also exist for primary hepatocyte cultures, tracheal organ cultures, and colon and mammary epithelium cultures.

In vitro evaluation of chemopreventive compounds should also involve screening for inhibition of transformation in the newer experimental cell systems where the molecular biology of transformation is beginning to be understood. These systems have provided one of the most far-reaching developments in cancer research by the unification of results from four distinct areas of investigation into a single comprehensive theoretic framework. This framework integrates detailed observations of chromosomal alterations, specific transforming genes in oncogenic viruses (oncogenes), dominant transforming genes in tumor cell DNA, and growth control by growth factors with hormone-like activity. The availability of cell lines with known and defined oncogene sequences (that encode proteins whose functions can be measured and presumably modulated to affect the transformation outcome) will be of paramount value in screening the ability of specific chemoprevention agents to inhibit mechanisms of carcinogenesis.

In Vivo Screening

In vivo screening systems for detecting inhibitors of carcinogenesis may be derived from procedures used

to determine the carcinogenicity of chemicals. In order to test for inhibitors of carcinogenesis, a chemical carcinogen is selected and employed in the test system within a particular range of concentrations. The effect of an inhibitor is generally to produce a response that would be expected from having given a lower dose of the carcinogen than that actually administered in the system. The carcinogen dose selected must therefore lie on a portion of the dose-response curve that is sensitive to alterations in the dose.

In addition to the total dose of carcinogen administered over time, the absolute amount given at any one time is important to in vivo screening. Since it is desirable to observe the results of inhibition in a relatively short time frame, sensitive biologic systems may be employed for administration of large doses of the carcinogen. However, human exposures to carcinogens and inhibitors are of relatively low dose and long duration and animal systems reflective of these conditions are important in tumor inhibition studies. Systems may be made more sensitive to demonstration of inhibition by increasing the survival time of animals and decreasing the dose of carcinogen, so that the carcinogen does not overwhelm the inhibitory effect.

The different animal tumor models that act as testing systems for demonstrating carcinogenicity are also useful for showing inhibition of carcinogenesis. In animal systems, the cancer inhibitory effects of chemopreventive agents may be studied in several different ways. The timing and route of administration for both the carcinogen and the potential cancer inhibitor may be varied.

The chemopreventive agent to be tested for its cancer-inhibitory capacity may be added to the diet, or may be given by separate oral administration at precise times prior to oral administration of the carcinogen. Alternatively, both carcinogen and inhibitor may be added directly to the diet. In the mouse forestomach model, for example, both inhibitor and carcinogen come into direct contact with the gastrointestinal tract to effect target tissue.[46] Many inhibitors in this model must be administered prior to, or at the time of, carcinogen administration in order to demonstrate cancer inhibitory effects.

Other models for demonstration of inhibition of carcinogenesis involve oral administration of carcinogens that produce tumors at remote sites rather than directly in the gastrointestinal tract. In one such model, mammary tumor formation may be induced by oral administration of dimethyl benzanthracene (DMBA).[47] A single oral dose usually produces mammary tumors in mice. The system can therefore be made more sensitive to inhibition of carcinogenesis by decreasing the size of the single oral dose, so that the carcinogenic effect does not overwhelm the inhibitory capacity. In another model demonstrating inhibition of tumor production in remote sites, oral administration of DMBA or other carcinogens may also cause induction of pulmonary adenomas and carcinomas in sensitive strains of mice. A dose-response relationship for carcinogen and tumor induction has been established in this model.[48] The potential chemopreventive agent may be administered as a regular component of the diet or as discrete single doses at precise times prior to oral administration of the carcinogen in order to demonstrate cancer inhibition.

Alternatively, some carcinogens that are administered subcutaneously to produce tumors in the gastrointestinal tract may also be inhibited by oral administration of the chemopreventive agent. 1,2-dimethyl-hydrazine (DMH) may be administered subcutaneously to rodent species in order to produce carcinomas of the large bowel.[49] In this model, the chemopreventive agent may be administered either through the diet or by separate oral administration before or after exposure to DMH. The carcinogen requires metabolic activation in the skin, and its action at the gastrointestinal tract is inhibited by chemopreventive agents given orally. Other direct-acting carcinogens, given intrarectally to produce rectal tumors, and not requiring metabolic activation in the skin or mucosa, may also be inhibited by oral chemopreventive agents.

Finally, carcinogen-induced epidermal neoplasia in the mouse is a model that involves topical administration of both carcinogen and chemopreventive agent to demonstrate cancer inhibition.[50] This model affords the ability to attain high local concentrations of the inhibitor while avoiding systemic toxicity. Many carcinogenic processes and inhibitory mechanisms that rely on systemic metabolic activation are not demonstrable in this system.

The above in vivo models demonstrate the characteristics of chemopreventive agents that appear to act by inhibition of tumor initiation. These agents may be administered just prior to, or at the time of, carcinogen exposure. They may act to protect target tissues in which both the carcinogen and the inhibitor are present.

Demonstration of inhibition of cancer promotion in vivo is complex, and requires selection of an appropriate spontaneous or carcinogen-induced animal tumor model, administration of a suitable cancer promoting agent, and administration of the potential cancer chemopreventive agent. Selection and evaluation of potential cancer chemopreventive agents for determination of efficacy may be accomplished through in vitro and in vivo screening systems discussed above. However, establishment of safety of potential cancer chemopreventive agents is also required prior to performance of human clinical trials.

Establishment of Safety

Determination of the suitability of a potential chemoprevention agent for clinical study requires preclinical evaluation of its toxicity and pharmacokinetic profile in animals. The acute, subacute/subchronic, and chronic toxicity of potential cancer chemoprevention agents may be determined in rodent species or dogs. Short-term studies and lifetime studies of toxicity, as well as multigenerational studies of teratogenicity and fetotoxicity, may be conducted on agents given by the oral route. Characterization of short-term toxicity involves acute toxicity and 13-week subchronic toxicity. End points are established with outcomes determined by gross necropsy, histopathologic study, and clinical laboratory examinations.

The relatively low level of toxicity that may be tolerated for cancer chemopreventive agents creates a special challenge for clinical pharmacology. Potential target populations for cancer chemopreventive agents consist of individuals at some risk of cancer, but without active disease. Therefore, the tolerable toxicity levels of chemopreventive agents are low in comparison to those of chemotherapeutic agents used in cancer therapy, making careful monitoring of toxicity a critical component of studies on these agents. Likewise, since cancer chemopreventive agents have the potential of being administered to women of childbearing age, teratogenicity is also important. Male-related reproductive toxicity may also be determined as a component of short-term toxicity studies or multigenerational studies.

The preclinical studies on chemopreventive agents discussed above may be used to establish efficacy in the presence of known toxicity, to determine toxicity of compounds with known efficacy, and to optimally adjust the therapeutic index (efficacy/toxicity) for cancer chemoprevention.

Clinical Studies

After adequate information on an agent's pharmacokinetics and toxicity is determined in animals, phase I clinical studies can be conducted in humans. Phase I clinical studies represent the first occasion that an agent is introduced into humans and emphasizes elucidation of the safety of the agent by defining the pharmacokinetic, toxicologic, and pharmacologic parameters associated with human use.

Phase I studies generally employ small numbers of healthy adults as study subjects. Their primary goal is to determine a safe starting dose to be used in phase II and phase III clinical trials. Where established, the recommended daily allowances (RDAs) for micronutrients may be taken as general guidelines for a starting human dose. However, they are no substitute for phase I clinical studies. While the RDAs are established on the basis of avoidance of nutritional deficiency states, higher doses, which may border on toxicity, may be required in order to optimize the cancer inhibitory effects. Therefore, RDAs relate to establishment of the lower limits for micronutrient intake, and phase I clinical studies are focused on setting upper limits for these agents.

Once phase I studies are complete, phase II and phase III studies are then conducted to further evaluate safety and to determine efficacy in clinical situations. These studies involve the use of control groups and larger target populations, in which rare events relating to the safety of the agent may be manifest. Larger target populations may be selected through identification of appropriate cancer risk groups.

Identification of Cancer Risk Groups

Selection of a suitable population for human intervention trials depends on the stage of carcinogenesis that the potential chemopreventive agent is expected to inhibit, the specific cancer under study, the age-specific incidence curve for the cancer, and the age of the study population.

Other information such as demographic variables (race, sex, socioeconomic status), occupational history, life-style, or medicopathologic factors specific to the cancer site of interest must also be considered.[51] Additional factors that may be relevant to the conduct of clinical trials include competing causes of death and level of effort required to assemble a study population of sufficient size. Compliance of individuals to the chemopreventive regimen in the trial must

also be taken into account. For example, age or other factors may influence the ease of assembly of a study population and the compliance to the chemopreventive regimen of individuals in the study.[52]

The population selected for an intervention trial should include individuals at sufficiently high risk, so that the number of cancer end points of interest will occur over a relatively short time period. If such a population can be identified, the need for massive sample sizes to properly test the hypothesis proposed in the study may be reduced. For example, in the trial of a potential chemopreventive agent for inhibition of bronchogenic lung cancer, selection of a population of elderly men with long-term smoking histories, whose bronchial mucosa had undergone squamous metaplasia, would yield a more rapid result in a controlled clinical trial than would a trial of an equal number of lower risk individuals.

On the other hand, high risk may overwhelm the potential effects of cancer chemopreventive agents. If carcinogenesis may occur through a number of different pathways, due to exposure to different cancer risk factors, the protective effect of any single cancer inhibitor may not be equally great in blocking each or all of these pathways. This potential problem suggests the need for a broad approach to human clinical trials, including studies of populations at "normal" risk for cancer as in the clinical trial currently being conducted by Hennekens.[53]

Measurement of the end point(s) in clinical trials of cancer chemoprevention agents is usually accomplished through the diagnosis of invasive cancer or documentation of cancer death. Another possible end point is the development of preneoplastic lesions that can be identified through various screening modalities. The advantages for the use of preneoplastic lesions, instead of invasive cancer, as end points include a shorter follow-up period to attainment of the end point, and the potential for a greater number of cases being diagnosed, including multiple end points in a single individual. Use of preneoplastic lesions as end points also permits conclusion of the trial prior to the development of invasive cancer in individuals if the chemopreventive agent appears effective. However, this type of end point has limitations, since reliable technology is not yet available for the determination of many "preneoplastic" lesions. Furthermore, the relationship of many lesions classified as "preneoplastic" to eventual development of cancer is also unclear for some cancer sites. The use of a precancerous lesion as an end point in a cancer clinical trial will therefore depend partially on the underlying model of carcinogenicity employed and the multistage nature of cancer development at a particular site. Further research is needed on potential uses of preneoplastic lesions as end points in chemoprevention trials. Biomarkers of exposure to carcinogens may be useful in the conduct of such trials.

Conduct of Controlled Clinical Trials

The potential toxicity of the cancer chemopreventive agent used in a controlled clinical trial should be balanced against the risk of cancer among high risk populations. In populations with a high risk of exposure to carcinogens, the use of a cancer chemopreventive agent must demonstrate a potential benefit that outweighs the risk of toxicity. In populations with a normal risk of exposure to chemical carcinogens, the potential chemopreventive agent should have no, or limited, toxicity in humans.

It should be kept in mind that no inhibitor of carcinogenesis is likely to be sufficiently efficacious that increasing the dose of the chemopreventive agent would have a greater effect on cancer prevention than would decreasing the exposure to the carcinogen. For example, it is unlikely that administration of any micronutrient with cancer chemopreventive activity would reduce lung cancer rates more than would prevention or cessation of smoking. However, if a number of different risk factors may cause a particular cancer, and the cancer chemopreventive agent inhibits a final common pathway for carcinogenesis at that site, then theoretically the effects of a potential chemopreventive agent may be greater than elimination of any one risk factor.

Potential Applications of Chemopreventive Agents

Research on population applications of potential chemopreventive agents may be conducted with the objective of community intervention. Successful completion of phase II and III clinical testing may lead to implementation of large-scale projects designed to test the feasibility of community interventions. Adequate attention must be given to such issues as product acceptability, storage requirements, dosing intervals, and product cost early in the process of chemopreventive agent development. Otherwise, these factors may adversely effect feasibility of a large-scale intervention or compliance level among the general population.

CONCLUSION

Current investigation on micronutrients, and their synthetic analogues, as potential inhibitors of carcinogenesis appears promising. Identification of chemopreventive agents offers the potential for prevention of cancer through supplementation of the human diet. This approach offers the possibility for major benefits in the public health. Clinical pharmacology has an important role in the identification of cancer chemopreventive agents that may substantially decrease the risk of cancer in human populations.

The authors wish to thank Drs. William DeWys and Gladys Block for reviewing the manuscript and for their helpful comments.

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